Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3063–3066

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Novel Nucleotide Phosphonate Analogues with Potent Antitumor Activity

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Received 24 May 2002; accepted 5 August 2002

Abstract—We have identified several nucleotide phosphonates demonstrating in vitro antiproliferative activity in several human cancer cell lines with IC_{50} values in the μM range. The synthesis as well as structure–activity relationship are described.
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Nucleotide phosphonates, which are characterized by a highly stable carbon–phosphorous bond between the nucleoside and the phosphate moiety, have a broad spectrum of antiviral activity but only a few have been reported showing antitumor activity.[1] Upon intracellular conversion to the active mono- and diphosphates by cellular kinases, they are incorporated into DNA during replication, or repair, leading to termination of DNA chain elongation. Acyclic nucleotide analogues, such as PMEG, show promise as cancer chemotherapeutic agents as they inhibit the growth of a wide range of solid tumor cell lines and induce cells to undergo apoptosis after blocking them in the S phase of the cell cycle.[2] Recently we have reported a novel class of tetrahydrofuran phosphonates of which the *cis* and *trans* guanine analogues (**1a** and **1b**) (Fig. 1), showed potent HCMV activity[3,4] and cytotoxicity.[5] The stereochemistry at the carbon linking the tetrahydrofuran to guanine was found to be crucial for biological activity since the corresponding enantiomers of **1a** and **1b** were found to be inactive. These findings prompted us to further explore the potential of these agents as cytotoxics. In this paper, we describe the synthesis and establish a structure–activity relationship (SAR) profile of the tetrahydrofuran phosphonate leads **1a** and **1b**.

The synthesis of **1a** and **1b** has been described in a previous paper.[3] We were interested in evaluating different substituted tetrahydrofuran derivatives of phosphonates **1a** and **1b**. Our initial biological results on the first set of hydroxylated analogues (**6a** and **6b**), which showed **6a** as having superior biological activity to **6b** prompted us to focus our efforts on evaluating analogues of **6a** (Table 1). Compound **3** is a key intermediate that would give us access to several of the 3′-substituted compounds (Scheme 1). Compound **2**, which was synthesized in according to literature procedures,[6] was converted to the MEM derivative by heating with 2-methoxyethanol and catalytic amount of pTSA at 60 °C. The alcohol was converted to the silylated derivative **3** and the phosphonate group was introduced by a Lewis acid catalyzed Arbuzov reaction. Thus, treatment of **3** with triisopropylphosphite in dichloromethane at −10 °C in the presence of titanium tetrachloride gave the phosphonates **4a** and **4b** in a 4.4:1 mixture of *cis* and *trans* isomers which were separated by column chromatography.[7] Phosphonates **4a** and **4b** were debenzoylated and converted to their corresponding mesylates. The crude mesylates were added to a solution of 2-amino-6-chloropurine and cesium carbonate[8] in DMF, which had been previously heated at

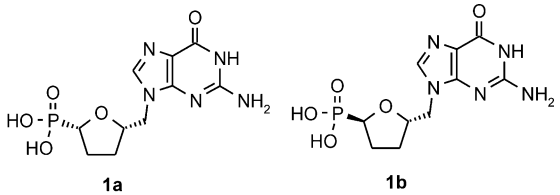

Figure 1. Cytotoxicity as measured in MRC-5 cell line.[3]

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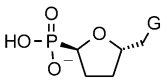
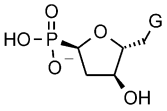
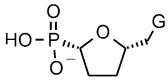
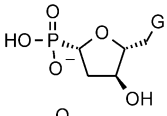
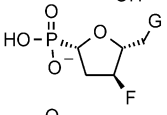
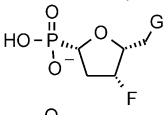
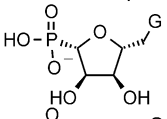
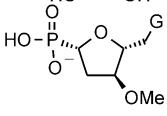
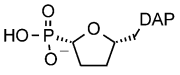
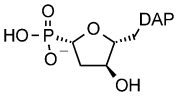
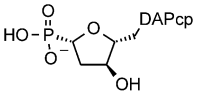
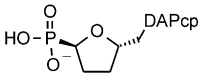
PII: S0960-894X(02)00679-0

100 °C for 1 h. The reaction mixture was heated at 100 °C for 16 h affording **5a** and **5b** in moderate yields. The phosphonate esters were deprotected by treatment with excess bromotrimethylsilane[9] followed by hydrolysis of the resulting trimethylsilyl ester along with the TBDMS ether, and the chloropurine was converted to guanine by refluxing in 10% aqueous HCl. The solution was basified with ammonia and purified by HPLC or by eluting through a charcoal column. The pure products were lyophilized to give the final compounds **6a** and **6b** as the ammonium salts.

Table 1. In vitro activities (IC_{50} in μM)[a] in human solid tumor cell lines[b] measured in a [^{3}H]-thymidine incorporation assay[c]

Compd[d]	H-460	MCF-7	SF-268
1b 	62–64	>100	>100
6b 	19–30	50–53	11–13
1a 	34–40	62–67	>100
6a 	0.28–0.53	0.33–0.67	0.12–0.14
14a 	20–44	98–>100	82–>100
17a 	5.0–7.1	>10–12	11–44
20a 	>100	>100	>100
10a 	4.5–7.6	2.6–5.6	1.9–2.4
23b 	30–57	41–52	>100
22a 	0.44–0.71	0.96–1.4	0.55–1.2
24a 	13–20	40–48	17–28
25b 	>100	>100	>100

[a] $n = 2$, in triplicate.
[b] Lung carcinoma, breast carcinoma, central nervous system tumor.
[c] Detailed experimental conditions have been described previously.[5]
[d] Compounds are as ammonium salts.

The *O*-methoxy derivative (**10a**) was prepared by installing the 3′-methoxy group at the start of the synthesis by reacting **2** with methyl iodide in the presence of silver (I) oxide (Scheme 1). The methylated product was converted to the MEM derivative (**7**), which was transformed into phosphonates **8a** and **8b** in a 5:1 ratio. Base coupling with **8a** and deprotection, following the procedure as described above, afforded **10a** in modest yield.

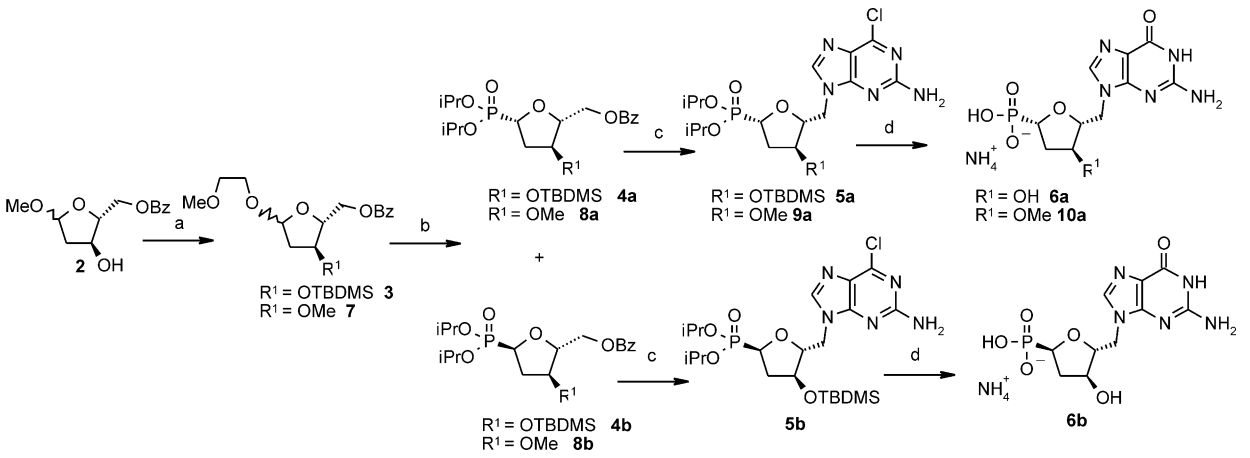
Both the 3′*R* and 3′*S* fluoro derivatives of **1a** were prepared starting with **11a** (Scheme 2), which was obtained from **4a** by acid hydrolysis. For the preparation of the 3′*S* isomer, the stereochemistry of the 3′-hydroxyl group of **11a** was inverted under Mitsunobu conditions. Hydrolysis of the resulting benzoate, followed by protection of the primary hydroxyl with a trityl group, gave **12a** in 65% yield over three steps. Fluorination with DAST[10] proceeded in 27% yield with the desired stereochemistry.[11] The trityl group was removed using acetic acid and the resultant product converted to the mesylate **13a**. Base coupling and deprotection as described in Scheme 1 afforded the final compound **14a** in 42% yield. To prepare the 3′*R* isomer, alcohol **11a** was debenzoylated and protected with a trityl group using standard procedures. The free 3-hydroxyl of **15a** was displaced by fluoride using DAST giving the *R* isomer in 31% yield. Acid hydrolysis and conversion to the mesylate gave **16a**, which upon base coupling and deprotection as previously described afforded the final compound **17a**.

The bis-hydroxylated compound (**20a**) was prepared from known furanose **18** (Scheme 3).[12] The phosphonate group was installed by the Lewis acid catalyzed Arbuzov reaction using TMSI at −78 °C. The use of TMSI[13] as a Lewis acid gave a 6:1 ratio of *cis* and *trans* phosphonates. The *cis* isomer was deacetylated and converted to the mesylate **19a**, upon which base coupling and deprotection afforded **20a** in low overall yield.

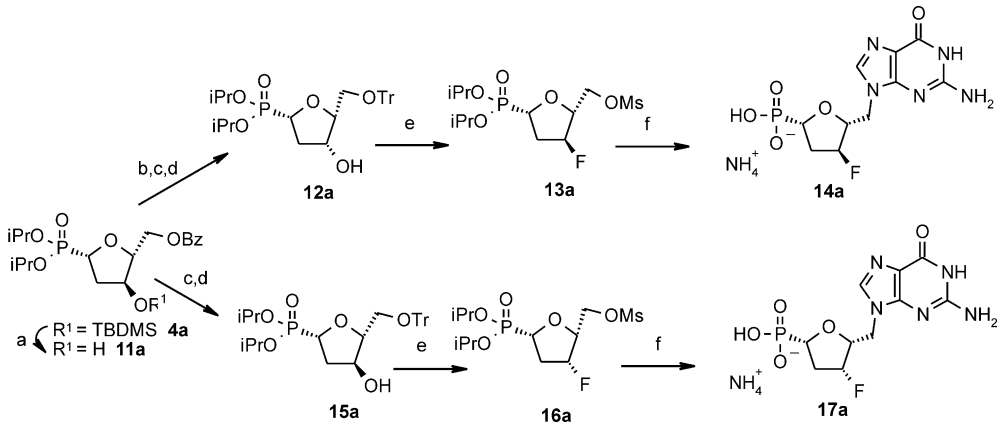
Upon consideration of the potencies of **6a** and **1b** (Table 1), we also prepared the diaminopurine (DAP) and *N6*-cyclopropyldiaminopurine (DAPcp) analogues which have been reported in the literature as potential prodrugs of the guanine derivatives.[2] The chloropurines (**5a** and **21b**,[3] Scheme 1) were converted to the diaminopurine analogues **22a** and **23b** by treatment with ammonia in ethanol at 100 °C followed by deprotection of the phosphonate esters (Scheme 4).[3]

Reacting the chloropurine derivatives with neat cyclopropylamine at 80 °C in a sealed tube for 16 h and deprotection of the phosphonate esters afforded the N^6-cyclopropyldiaminopurine analogues **24a** and **25b** in good yields.

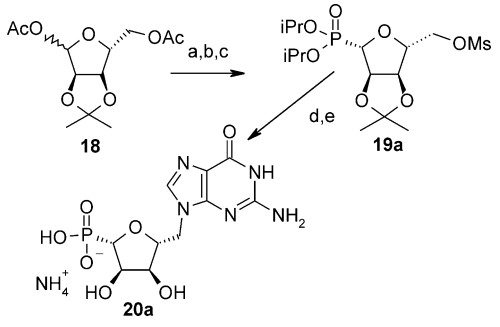
The antiproliferative activity of the tetrahydrofuran phosphonates was measured using a (^{3}H)-thymidine incorporation assay in various cell lines.[14] Comparing the activities of the *trans* guanine analogue (**1b**) to its corresponding 3′-hydroxy derivative (**6b**), there is a slight enhancement in activity (Table 1) probably due to



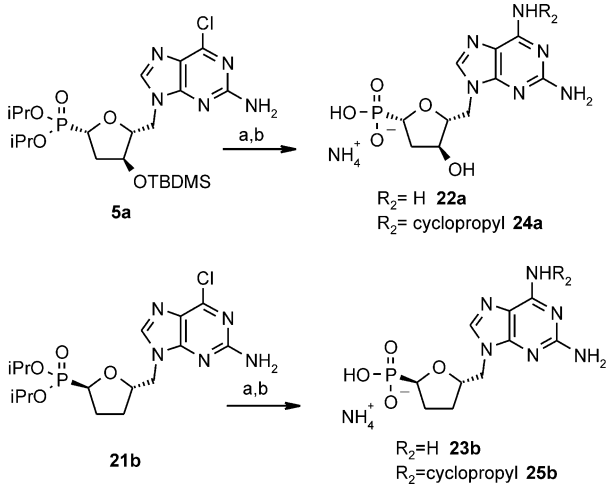
Scheme 1. (a) **3**: (i) $CH_3OCH_2CH_2OH$, pTSA, 92%; (ii) TBDMSCl, imidazole, DMF, 73%; **7**: (i) CH_3I, Ag_2O, DMF, 95%; (ii) $CH_3OCH_2CH_2OH$, pTSA, 83%; (b) $TiCl_4$, triisopropylphosphite, CH_2Cl_2 65% **4a**, 15% **4b**, 45% **8a**, 9% **8b**; (c) **5a**: (i) K_2CO_3, MeOH, 89%; (ii) MsCl, TEA, CH_2Cl_2, 0 °C; (iii) 2-amino-6-chloropurine, Cs_2CO_3, DMF, 100 °C, 47%; **5b**: (i) K_2CO_3, MeOH, 95%; (ii) MsCl, TEA, CH_2Cl_2, 0 °C; (iii) 2-amino-6-chloropurine, Cs_2CO_3, DMF, 100 °C, 40% (over two steps); **9a**: (i) K_2CO_3, MeOH, 85%; (ii) MsCl, TEA, CH_2Cl_2, 0 °C; (iii) 2-amino-6-chloropurine, Cs_2CO_3, DMF, 100 °C, 36% (over two steps); (d) (i) TMSBr, rt; (ii) 10% HCl (aq), reflux; (iii) NH_4OH, charcoal column, 72% **6a**, 76% **6b**, 95% **10a**.



Scheme 2. (a) MeOH, HCl (aq), 42%; (b) benzoic acid, PPh_3, DEAD, ether; (c) K_2CO_3, MeOH; (d) TrCl, Py, 65% **12a** (over three steps), 40% **15a** (over two steps); (e) **13a**: (i) DAST, Py, 27%; (ii) 80% AcOH(aq), 95%; (iii) MsCl, TEA, CH_2Cl_2, 0 °C; **16a**: (i) DAST, Py, 31%; (ii) 80% AcOH (aq), 77%; (iii) MsCl, TEA, CH_2Cl_2, 0 °C; (f) **14a**: (i) 2-amino-6-chloropurine, Cs_2CO_3, DMF, 100 °C, 42%; (ii) TMSBr, rt; (iii) 10% HCl (aq), reflux; (iv) NH_4OH, charcoal column, 98% (over three steps); **17a**: (i) 2-amino-6-chloropurine, Cs_2CO_3, DMF, 100 °C, 32%; (ii) TMSBr, rt; (iii) 10% HCl (aq), reflux; (iv) NH_4OH, charcoal column, 76% (over three steps).



Scheme 3. (a) TMSI, triisopropylphosphite, CH_2Cl_2, 54%; (b) $NaOCH_3$, MeOH, 99%; (c) MsCl, TEA, CH_2Cl_2, 0 °C; (d) 2-amino-6-chloropurine, Cs_2CO_3, DMF, 100 °C, 21% (over two steps); (e) (i) TMSBr, rt; (ii) 10% HCl (aq), reflux (iii) NH_4OH, charcoal column, 56%.



Scheme 4. 21a, **22b**: (a) NH_3, EtOH, 100 °C; (b) (i) TMSBr, rt; (ii) NH_4OH, charcoal column, 75%, 82% (over two steps); **23a**, **24b**: (a) cyclopropyl amine, 80 °C, sealed tube; (b) (i) TMSBr, rt; (ii) NH_4OH, charcoal column, 61%, 62% (over two steps).

the hydroxyl group. On the other hand, the 3′-hydroxy analogue of the *cis* guanine derivative (**6a**), displayed a dramatic 100-fold increase in activity compared to **1a**. The fluoro analogues showed an interesting activity profile; the 3′*S* isomer (**14a**) was inactive whereas the 3′*R* isomer (**17a**) displayed a slight enhancement in activity in comparison. The bis-hydroxylated (**20a**) exhibited no antiproliferative activity indicating that substitution on the 2′-position may not be tolerated. By methylating the 3′-position of (as in **10a**), one is still able to retain biological activity. Diaminopurines **23b** and **22a** also retained activity of guanine analogues **1b** and **6a**, whereas the N^6-cyclopropyldiaminopurine analogues **24a** and **25b** were inactive. These results suggest that the N^6-cyclopropyldiaminopurines are not converted to the guanine derivatives.

We have identified several potent phosphonate nucleotides in vitro. The in vivo efficacy and the mechanism of action of these compounds are currently being examined and will be presented in due course.[5]

Acknowledgements

The authors would like to thank Thérèse Godbout for her help in the preparation of this manuscript.

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7. All attempts to assign the relative stereochemistry using NOE experiments failed since adequate separation of the relevant proton signals of various intermediates could not be achieved. We assigned the major isomer, **4a**, as having the *cis* configuration since we believe the approach of the large phosphite would be opposite to the bulky TBDMS group. Under identical Arbuzov reaction conditions, no stereochemical bias was observed with compounds lacking 3′-substituants, such as **1a** and **1b**.
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11. The stereochemistry of **14a** and **17a** was assigned based on a comparison of $J(C_4,F)$ values of the precursors with those of structurally similar compounds reported in the literature: (a) Mikhailopulo, I. A.; Sivets, G. G. *Helvetica Chimica Acta* **1999**, *82*, 2052. (b) Fleet, G. W. J.; Son, J. C. *Tetrahedron Lett.* **1987**, *28*, 3615.
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14. All cell lines were adapted in RPMI culture media. The cells are treated for 72 h at 37 °C and 5% CO_2 with the drugs. Methyl ^{3}H-thymidine (0.5 μCi) is added for the last 16 h. The cells were harvested on a fiber glass filter mat and counted on a Beta counter.